

Rohatgi provides support that both fluorescein and tetramethylrhodamine are capable of dye-stacking, Wei provides support that fluorescein and tetramethylrhodamine attached to a peptide can interact to "essentially" self-quench the fluorescence groups, and Tsien provides further support that hydrophobic donor and acceptor fluorophores can stack when separated by a short flexible linker in a polar solvent. One skilled in the art would conclude that fluorescein and tetramethylrhodamine attached to a peptide substrate in an aqueous buffer would inherently stack. The Examiner states that resonance energy transfer and dimerization is not mutually exclusive. As support, the Examiner cites Wei as teaching that quenching efficiency of a dual labeled peptide is probably due to both dimerization and energy transfer and cites Tsien as teaching that as dyes in a dual labeled substrate diffuse away from each other stacking and energy transfer are disrupted.

The Examiner states that one skilled in the art would reasonably conclude that fluorescein and tetramethylrhodamine attached to a peptide substrate in aqueous buffer as taught by Garman would inherently be stacked when exhibiting dye quenching. The Examiner further states that dye dimerization and resonance energy transfer are not mutually exclusive and cites Wei and Tsien in support of this statement.

The Examiner cites *In re Best, Bolton, and Shaw*, 195 USPQ 430, 431 (CCPA 1977), for the following: "Mere recitation of newly-discovered function or property, inherently possessed by things in the prior art, does not cause a claim drawn to those things to distinguish over the prior art; Patent Office can require applicant to prove that subject matter shown to be in prior art does not possess characteristic relied on where it has reason to believe that functional limitation asserted to be critical for establishing novelty in claimed subject matter may be inherent characteristic of prior art".

The Examiner concludes that Applicant has not provided any evidence that the fluorescent groups attached to the peptide substrate of Garman would not inherently stack under the conditions taught in Garman and that Applicant needs to provide convincing evidence to the contrary.

Applicants respectfully draw the Examiner's attention to subsequent case law, including *Ex Parte Skinner* that acknowledges the language of *In re Best, Bolton, and Shaw* (as also disclosed in *In re Swinehart*, 169 USPQ 226 (CCPA 1971)), but goes on to state:

"Nevertheless, before an applicant can be put to this burdensome task, the examiner must provide some evidence or scientific reasoning to establish that the functional limitation is an inherent characteristic of the prior art."

Applicants submit that the Examiner cannot meet this burden because the substrates of Garman cannot, by definition, inherently dimerize. As stated in *Continental Can Co. USA v. Monsanto Co.*, 20 USPQ2d 1747, 1749:

"To serve as an anticipation when the reference is silent about the asserted inherent characteristic, such gap in the reference may be filled with recourse to extrinsic evidence. Such evidence must make clear that the missing descriptive matter is **necessarily present** (emphasis added) in the thing described in the reference, and that it would be so recognized by persons of ordinary skill".

The *Continental Can* court further cites *In re Oelrich*, 212 USPQ 323, 326 (CCPA 1981) as providing:

"Inherency, however may not be established by probabilities or possibilities. The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient. If, however, the disclosure is sufficient to show that **the natural result flowing from the operation as taught** (emphasis added) would result in the performance of the questioned function, it seems to be well settled that the disclosure should be regarded as sufficient."

Garman does not disclose or teach dimerization. It discloses only energy transfer. Energy transfer involving two fluorophores requires, by definition, that the donor molecule quench the fluorescence of the acceptor molecule, while the fluorescence of the donor molecule is **not** quenched. Dimerization requires that the fluorescence of **both** interacting molecules be quenched. Because a fluorescent molecule cannot be both quenched and not quenched at the same time, Applicants maintain their position that dimerization and energy transfer are mutually exclusive. However, this is not say that it is impossible for two dye molecules on a substrate to undergo dimerization and energy transfer at different times, depending on factors and conditions such as whether the substrate can achieve the different configurations needed to provide the appropriate dye alignments for energy transfer and dimerization. The possibility of both dimerization and energy transfer is mentioned in Wei, which is cited by the Examiner. See Wei at p. 1503, col. 1. The Examiner also cites Tsien, at col. 18, lines 14-43, as disclosing the same thing. However, Tsien actually talks about separate energy transfer and dimerization situations (see, e.g., line 37 "In either case").

Nevertheless, even if it is possible for a fluorogenic substrate to undergo both dimerization and energy transfer, it does not follow that a fluorogenic substrate that undergoes energy transfer will **necessarily** undergo dimerization or that dimerization is the

**natural result** flowing from energy transfer fluorogenic substrates. Because the substrates of Garman are energy transfer substrates, they do not inherently meet the requirements of the claims of the present invention. The claims of the present invention require that the dye groups be drawn together by free energy attractions such that they self-quench due to dye dimerization or stacking. This type of interaction is contrary to the energy transfer interaction of Garman.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP 2131 (citing *Verdegaal Bros. V. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). Because Garman does not disclose expressly, or inherently, even with the support of Rohatgi, Wei, and Tsien, every element of the presently claimed invention, Applicant(s) submit that the cited references cannot support a 35 U.S.C. 102(b) rejection and respectfully requests that the rejection be withdrawn.

### 35 U.S.C. §103 Obviousness Rejections

According to MPEP 2142, to establish a case of *prima facie* obviousness, three basic criteria must be met: 1) there must be some suggestion or motivation, either in the references or generally known to one of skill in the art, to modify or combine the reference teachings, 2) there must be reasonable expectation of success, and 3) the prior art references must teach or suggest all the claim limitations. The ability to modify the method of the references is not sufficient. The reference(s) must provide a motivation or reason for making the changes. *Ex parte Chicago Rawhide Manufacturing Co.*, 226 USPQ 438 (PTO Bd. App. 1984).

### 35 U.S.C. 103(a) – Garman, Rohatgi, Wei, and Tsien

Claims 1, 3-4, 6-8, 10, 12-13, and 15-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Garman et al. (GB 2278356) (hereinafter "Garman") as supported by Rohatgi et al. (J. Phys. Chem (6/1966) vol. 70 (6), pages 1695-1701) (hereinafter "Rohatgi"), Wei et al. (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506) (hereinafter "Wei"), and Tsien (U.S. Pat. No. 5,741,657) (hereinafter "Tsien").

The Examiner relies on the same arguments as presented for the 102(b) rejection.

Applicants respectfully submit that the references cannot support a case of *prima facie* obviousness as to the claims because, among other possible reasons, the cited references do not provide a motivation or suggestion to provide a substrate having dimerizing pairs of fluorescent

moieties. The purpose of Garman was to provide an improved method of preparing a fluorescence resonance energy transfer substrate. *See, e.g.*, Garman at p. 2, 3<sup>rd</sup> full paragraph. As is described in the present specification at p. 4, line 17 to p. 5, line 10, energy transfer is a different type of quenching from dimerization. Accordingly, there is no motivation to substitute dimerizing moieties for the energy transfer moieties of Garman. Furthermore, because the substrates of Garman provide a configuration that results in energy transfer, there could be no reasonable expectation that dimerization could be achieved with the substrates of Garman, especially since Garman already uses fluorescein and rhodamine, which the Examiner cites as a dimerizing pair. At most, based on the teachings of the cited references, it would be obvious to try to invent a substrate that would allow dimerization. However, obvious to try is not the proper standard for obviousness. *In re Dow Chemical Co.*, 5 USPQ2d 1529 (Fed. Cir. 1988). For these reasons, Applicants submit that the cited references will not support a 103(a) rejection of the claimed invention and request that the rejection be withdrawn.

35 U.S.C. 103(a) – Garman, Rohatgi, Wei, Tsien, and Komoriya

Claims 1-8 and 10-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Garman et al. (GB 2278356) (hereinafter “Garman”) as supported by Rohatgi et al. (J. Phys. Chem (6/1966) vol. 70 (6), pages 1695-1701) (hereinafter “Rohatgi”), Wei et al. (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506) (hereinafter “Wei”), Tsien (U.S. Pat. No. 5,741,657) (hereinafter “Tsien”), and in view of Komoriya et al. (US 5,714,342) (hereinafter “Komoriya”).

The Examiner maintains that Garman teaches dimerizing dyes and that it would have been obvious to combine the teachings of Garman, Rohatgi, Wei, Tsien, and Komoriya.

Applicants respectfully submit that the references cannot support a case of prima facie obviousness as to the claims for the same reasons as stated in the response to the 103(a) rejection based on Garman, Rohatgi, Wei, and Tsien. For these reasons, Applicants submit that the cited references will not support a 103(a) rejection of the claimed invention and request that the rejection be withdrawn.

35 U.S.C. 103(a) – Garman, Rohatgi, Wei, Tsien, Komoriya, and Heath

Claims 9 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Garman et al. (GB 2278356) (hereinafter “Garman”) as supported by Rohatgi et al. (J. Phys. Chem. (6/1966) vol. 70 (6), pages 1695-1701) (hereinafter “Rohatgi”) and Wei et al. (Anal.

Chem (5/1994), vol. 66 (9), pages 1500-1506) (hereinafter "Wei"), Tsien (U.S. Pat. No. 5,741,657) (hereafter "Tsien"), in view of Komoriya et al. (US 5,714,342) (hereinafter "Komoriya"), as applied to claims 1-8 and 10-18 above, and further in view of Heath, Jr., et al. (US 5,235,039) (hereinafter "Heath, Jr.").

The Examiner maintains that Garman teaches dimerizing dyes and that it would have been obvious to have combined the teachings of Garman, Rohatgi, Wei, Tsien, Komoriya, and Heath.

Applicants respectfully submit that the references cannot support a case of prima facie obviousness as to the claims for the same reasons as stated in the response to the 103(a) rejection based on Garman, Rohatgi, Wei, and Tsien. For these reasons, Applicants submit that the cited references will not support a 103(a) rejection of the claimed invention and request that the rejection be withdrawn.

35 U.S.C. 103(a) – Garman, Rohatgi, Wei, Tsien, and Manafi

Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Garman et al. (GB 2278356) (hereinafter "Garman") as supported by Rohatgi et al. (J. Phys. Chem (6/1966) vol. 70 (6), pages 1695-1701) (hereinafter "Rohatgi") and Wei et al. (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506) (hereinafter "Wei"), Tsien (U.S. Pat. No. 5,741,657) (hereafter "Tsien"), and in view of Manafi et al. (Microbiol. Reviews (9/1991), vol. 55 (3), pages 335-348) (hereinafter "Manafi").

The Examiner maintains that Garman teaches dimerizing dyes and that it would have been obvious to have combined the teachings of the references.

Applicants respectfully submit that the references cannot support a case of prima facie obviousness as to the claims for the same reasons as stated in the response to the 103(a) rejection based on Garman, Rohatgi, Wei, and Tsien. For these reasons, Applicants submit that the cited references will not support a 103(a) rejection of the claimed invention and request that the rejection be withdrawn.

**Objection**

Claim 20 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form.

Applicants have not rewritten claim 20 because they believe the parent claim of claim 20 is allowable.

In addition to the foregoing arguments, Applicants submit that a dependent claim should be considered allowable when its parent claim is allowed. *In re McCain*, 101 USPQ 411 (CCPA 1954). Accordingly, provided independent claims 1, 12 and 21 are allowed, all claims depending therefrom should also be allowed.

Based on the foregoing, it is submitted that the application is in condition for allowance. Withdrawal of the rejections under 35 USC 102 and 103 is requested. Examination and reconsideration of the claims are requested. Allowance of the claims at an early date is solicited.

Applicants and their attorney thank the Examiner for the telephone interview of February 1, 2002 during which the essence of the amendments and arguments contained herein were discussed. Examiner Marjorie Moran, attorney Melanie Gover, inventor Ai-Ping Wei, and 3M employee Dean Ersfeld were present at the interview. The Examiner is invited to contact Applicants' attorney if the Examiner believes any remaining questions or issues could be resolved.

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Respectfully submitted,

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**Version With Markings to Show Changes Made**

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1. (twice amended) A method of biological assay comprising:



providing an enzyme substrate comprising two or more fluorescence dye groups bound to a flexible peptide comprising one or more enzymatically cleavable bonds, the dye groups being [proximate such that] drawn together by free energy attractions such that [draw] the dye groups [together so as to] self-quench their fluorescence [of the dye groups, wherein self quenching of fluorescence of the dye groups is effected] by dye stacking or dimerization, and

- b) contacting said substrate with a substance being assayed to determine the presence of an enzyme capable of cleaving an enzymatically cleavable bond wherein the enzymatic cleaving of said cleavable bond[s] of the peptide will release the fluorescent dye groups from dye stacking, thereby producing an increase in fluorescence intensity and indicating the presence of said enzyme.

12. (twice amended) A protease substrate comprising a flexible peptide and including two fluorescence dye groups[, the dye groups being proximate such that] drawn together by free energy attractions [draw the dye groups together] so as to self-quench fluorescence of the dye groups by intramolecular dimerization or stacking.

21. (twice amended) An assay method of detecting a microorganism, which microorganism produces a characteristic enzyme, comprising:

- a) providing an enzyme substrate specific for said characteristic enzyme produced by said microorganism comprising two or more fluorescence dye groups bound to a flexible peptide comprising one or more bonds cleavable by said characteristic enzyme, the dye groups being [proximate such that] drawn together by free energy attractions [draw] such that the dye groups [together so as to] self-quench their fluorescence [of the dye groups, wherein self quenching of fluorescence of the dye groups is effected] by dye dimerization or stacking, and
- b) cleaving one or more of said cleavable bonds of the peptide by said characteristic enzyme to release the fluorescence dye groups from dye dimerization or stacking, thereby producing an increase in fluorescence intensity which indicates the presence of said microorganism.